Prognostic value of osteoprotegerin in acute heart failure

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Brief Summary

In a prospective study involving 338 patients, plasma levels of osteoprotegerin at discharge of a hospital admission for acute heart failure were associated with higher all-cause mortality or readmission for worsening heart failure during a 6-month period, independently of known morbimortality markers such as age, diabetes, ischaemic aetiology, BNP, NYHA class, renal function, C-reactive protein and the presence or severity of left ventricle systolic dysfunction.
ABSTRACT

Background: Osteoprotegerin (OPG) is promising as a predictor of adverse prognosis in patients with acute coronary syndromes and chronic heart failure. Its prognostic value in acute heart failure (AHF) is unknown. The aim of this study was to assess the prognostic value provided by serum OPG levels at discharge of an admission for AHF.

Methods: In a prospective study, we enrolled 338 patients consecutively admitted with AHF to the Internal Medicine Department of a tertiary care university hospital in Porto, between March 2009 and December 2010. OPG was measured using a commercial enzyme-linked immunosorbent assay, and analysed both as a continuous variable and categorized by quartiles. Patients were followed for up to six months after discharge to ascertain the occurrence of all-cause death or hospital readmission due to AHF.

Results: During follow-up, 119 patients died or were readmitted due to AHF. A graded increase in the risk of the combined endpoint was observed across quartiles of OPG. At 6 months, the cumulative risk of the endpoint was 25% for the first and 50% for the fourth quartile. The multivariable-adjusted risk of death or AHF hospitalization increased progressively across categories of OPG up to a statistically significant 2.44-fold increase in risk in the highest category (p value for linear trend=0.002), by 5% per 10 pg/mL increase in OPG.

Conclusions: Serum OPG was directly associated with a higher probability of death or readmission for AHF within 6 months, irrespective of other known prognostic markers, both in preserved and reduced ejection fraction.

KEYWORDS: osteoprotegerin; biomarker; acute heart failure; prognosis.
INTRODUCTION

Initially associated with bone mineral density regulation [1], a growing body of evidence suggests that osteoprotegerin (OPG) plays an important role as a mediator in vascular remodelling and atherosclerotic disease [2-4]. Accordingly, OPG has emerged as a promising biomarker of cardiovascular morbidity and mortality [5-11].

A member of the tumour necrosis factor (TNF) superfamily, OPG interacts with the receptor activator of nuclear factor \( \kappa B \) (RANK) and its ligand (RANKL), and also with the TNF-related apoptosis-inducing ligand (TRAIL), acting as a decoy receptor for both ligands and antagonizing their effects, i.e. cytokine release, monocyte transmigration and apoptosis [12-13]. Since persistent inflammation has emerged as an important mechanism in the development and progression of chronic heart failure (CHF) [14-16] and further motivated by the disappointing results from anti-TNF trials [17-18], the role OPG plays in the development and progression of heart failure has been the object of both basic and clinical research [19-21]. These data suggest that OPG is a useful predictor for mortality [22] and hospitalization for worsening heart failure [23], supporting the theory that OPG/RANK/RANKL axis may represent a new and promising target for heart failure therapy.

Compared to CHF, acute heart failure (AHF) is a far less understood clinical entity, with diverse clinical presentations and corresponding therapeutic approaches [24]. AHF is a major health problem worldwide and is the most frequent cause of hospital admission in patients older than 65 years, accounting for 60% of all expenditures of heart failure treatment [25-28]. Despite therapeutic interventions, AHF is still associated with an ominous prognosis [29-30]. So far, sparse data exists regarding the role OPG plays in AHF. Accordingly, in this study we investigated the...
prognostic value provided by OPG serum levels at discharge, in patients admitted to a tertiary-care hospital because of AHF.

MATERIAL AND METHODS

Study sample

In a prospective study on the prognosis of AHF, we enrolled all patients consecutively admitted with the diagnosis of AHF to the Internal Medicine Department of Hospital de São João, a public tertiary care university hospital in Porto, between March 2009 and December 2010. Heart failure diagnosis was based on the European Society of Cardiology criteria [24], and both reduced and preserved ejection fraction patients were included. Patients were eligible whether AHF was de novo or an exacerbation of CHF with an increase in at least one New York Heart Association (NYHA) functional class. Patients with an acute coronary syndrome and patients on chronic renal function replacement therapy were excluded. Thirty patients died during the index hospitalization. Of the 612 who were discharged alive, a consecutive subsample of 401 had OPG measured in serum collected before discharge. One patient was lost to follow-up and 62 further patients had missing data on variables included in the final multivariate model, leaving 338 for the current analysis.

Study design and procedures

Demographic and clinical data were recorded by the research team, based on interviews to the patients and on hospital records.
An echocardiogram was performed within 72 hours of admission. All images were obtained using standard ultrasound equipment (System 6, GE Vingmed, Horten, Norway) with a 2.5-MHz probe. Left ventricular ejection fraction was calculated by the biplane Simpson's method. Patients were classified as heart failure with preserved ejection fraction (HFpEF) or heart failure with reduced ejection fraction (HFrEF), using 45% as cut-off.

Fasting venous blood samples were collected between 8 and 9 a.m. on the last hospitalization day. Specimens were centrifuged for 10 minutes at 3000 × g within two hours after laboratory arrival. Glomerular filtration rate was estimated according to the Modification of Diet in Renal Disease (MDRD) formula [31]. Serum OPG (pg/mL) was assayed in 2013, in serum stored at -80°C, using enzyme-linked immunosorbent assay kits (MicroVue-Quidel Corporation, San Diego, USA). The lower limit of detection is 8 pg/ml, and the within-run and the run-to-run variation was 2.1%-3.5% and 4.2%-6.1%, respectively.

Patients received standard treatment according to the attending physicians, and the timing of discharge was at their discretion.

The endpoint in the analysis was all-cause death or readmission due to AHF. Patients were followed for up to six months after discharge. Information about the endpoint was obtained from hospital records and telephone contact with the patients.

_Ethical standards_

The study protocol conforms to the ethical principles of the 1964 Declaration of Helsinki and its later amendments, the local ethics committee approved the study and patients provided written informed consent prior to their inclusion in the study.
Statistical analysis

Continuous variables are presented as mean (standard deviation) if normally distributed or median (interquartile range) if skewed. Categorical variables are presented as proportions. The Kruskal-Wallis test was used to compare numerical variables and Pearson Chi-square test for categorical variables.

We used the Kaplan-Meier method to estimate the risk of all-cause death or readmission due to AHF within six months, according to quartiles of serum OPG. Cox regression models were used to quantify the association between serum OPG and the six-month risk of the endpoint. The potential confounders were selected departing from previous knowledge [32-34] to list variables expected to predict worse prognosis in this population, and then kept in the model if either they had a significant effect on the outcome or they changed the regression coefficient for the effect of OPG by more than 10%.

Statistical analyses were performed using Stata version 11.1 for Windows (StataCorp LP, College Station, TX). P values lower than 0.05 were considered statistically significant.

RESULTS

Overall, of the 338 patients with AHF, 33 (9.8%) had heart failure de novo and the remaining had a decompensation of CHF. There were 180 (53.2%) women; the average age was 76 years. All patients were Caucasian. Serum OPG ranged from 39.6
to 506.7 pg/ml, with a median value of 146.2 pg/ml. Table 1 depicts the patients’ characteristics by quartiles of serum OPG. Age increased strongly and significantly by 10 years, on average, from the first to the fourth quartile. OPG was also positively associated with NYHA class at discharge, with a 4-fold increase in the proportion discharged in NYHA class III-IV from the first to the fourth quartile. Serum BNP increased and estimated glomerular filtration rate decreased with the increase in OPG, both in a graded way. C-reactive protein showed a direct association with OPG. There were no important differences in the drug therapy prescribed at discharge according to OPG [Table 1]. OPG levels were not different in the 12 patients with moderate to severe aortic stenosis.

The combined endpoint all-cause death or hospital readmission for AHF was observed in 119 patients. As shown in Figure 1, after approximately 1 month post-discharge from the index hospitalization, the estimated survival curves separate and a graded increase in the risk of the endpoint is observed across the quartiles of OPG. At 6 months, the cumulative risk of the endpoint is 25% for the first and 50% for the fourth quartile.

When adjusting for potential confounders, the risk of death or AHF hospitalization increased progressively across categories of OPG up to a statistically significant 2.44-fold increase in risk in the highest category (p value for linear trend=0.002). The graded effect translated into a statistically significant increase in risk of the endpoint by 5% per 10 pg/mL of OPG [Table 2].

The association between OPG and the combined endpoint stratifying by preserved versus reduced ejection fraction is presented in Table 2. There was no significant difference in the prognostic effect of OPG between these groups (hazard ratio (95% confidence interval) = 1.04 (1.01-1.08) in HFpEF versus 1.06 (1.01-1.11) in HFrEF, p for interaction=0.792).
Sensitivity analyses

After exclusion of 126 patients whose decompensation was due to an infection, the hazard ratio for the composite endpoint was 1.99 (0.86-4.63), 2.04 (0.92-4.55) and 2.74 (1.20-6.25) across the 2nd, 3rd and 4th quartiles, respectively, compared to the first, p for trend=0.023.

When excluding 33 patients with de novo heart failure, the hazard ratio for the composite endpoint was 1.36 (0.72-2.56), 1.37 (0.74-2.56) and 2.13 (1.15-3.92) across the 2nd, 3rd and 4th quartiles, respectively, compared to the first, p for trend=0.014.

DISCUSSION

Plasma levels of OPG at discharge after hospital admission for AHF were related to higher any-cause mortality or admission for worsening heart failure during a 6-month period, independently of known morbimortality markers such as age, diabetes, ischaemic aetiology, BNP, NYHA class, renal function, C-reactive protein and the presence or severity of left ventricle systolic dysfunction.

OPG, formerly known as osteoclastogenesis inhibitory factor, was initially thought to be exclusively related to bone metabolism [1]. Nevertheless, it rapidly became a point of interest in all pathways where the handling of calcium can be disturbed, such as atherosclerosis and chronic renal failure [35-36]. Thus, the contribution of OPG to a more accurate risk stratification in the cardiovascular diseases continuum is being noted, with several studies associating OPG to the progression of
asymptomatic atherosclerosis and to the severity of coronary disease [7, 10, 37], and predicting a higher mortality and cardiovascular morbidity in patients with acute myocardial infarction [8-11, 38].

In the specific setting of HF, several biochemical pathways are activated (neurohumoral activation, oxidative stress, matrix remodelling, myocardial stress, myocyte injury, inflammation, renal dysfunction) working together, leading to the disruption and/or perpetuation of the syndrome and providing a myriad of biological substances potentially useful as biomarkers [39]. Acting as a decoy receptor for some pro-inflammatory cytokines, OPG reflects the inflammatory status as well as it protects cells (including myocytes) from apoptotic stimuli and is related to hemodynamic and neurohumoral disease severity [20]. Nevertheless, the results of two studies using cohorts derived from two major clinical trials in CHF that assessed its potential as a biomarker are somewhat discrepant from each other: OPG was associated with all-cause mortality and cardiovascular hospitalizations (despite modest significance) in a sub-analysis of the Gruppo Italiano per lo Studio della Sopravivenza nell’Infarto Miocardico – Heart Failure trial [22], whereas in the Controlled Rosuvastatin Multinational Trial in Heart Failure OPG levels were only related to hospitalization for worsening heart failure, not adding predictive information on what concerns to all-cause mortality or the combined end-point of cardiovascular death, nonfatal myocardial infarction or nonfatal stroke [23].

Concerning the more recent field of AHF, OPG was once previously evaluated as a prognostic tool but only in the specific setting of HFpEF [40]. Our study went beyond the item of ventricular function, showing results that support OPG as a useful marker for prognostic assessment within 6 months after an AHF hospital admission, irrespective of the left ventricle ejection fraction [Table 2]. Interestingly, as observed in the Kaplan-Meier curves for quartiles of serum OPG [Fig. 1], prognosis seems to be
well stratified only about one month after discharge from hospital, a situation not much
different from studies evaluating OPG after acute myocardial infarction [11, 37],
suggesting a similitude of mechanisms in these two settings, involving ischemia,
inflammation, myocardial fibrosis, matrix degradation and remodelling, and leading to
new or aggravated cardiac dysfunction [41].

Since OPG is a reliable indicator of the OPG/RANK/RANKL axis, and thereby, a
marker of inflammation, it could be elevated in infectious situations, a major problem
and a frequent cause of decompensation in the AHF setting. When excluding from the
analysis patients whose precipitant factor was an infection, the conclusions remained
the same.

Besides being a promising predictor of prognosis, and taking into account the
mechanisms represented by the OPG/RANK/RANKL axis, and the fact that OPG acts,
in fact, as a cell protector from pro-inflammatory cytokine aggression and apoptosis
induction, efforts are being made to a targeted intervention. Whereas there are some
promising results in treating postmenopausal osteoporosis and bone metastasis from
solid tumours using denosumab, a monoclonal antibody against RANKL [42-43], no
data is available concerning its clinical use in cardiovascular disorders, including heart
failure.

Strengths and limitations

Our medium-sized cohort of AHF patients admitted consecutively to an Internal
Medicine Department of a tertiary-care hospital, reflects the description of major
registries on the subject [29-30], representing an elderly population (75% older than 70
years), with about half of individuals being women (53%), diabetics (45%) or with heart
failure of ischaemic aetiology (49%), and having heart failure with preserved ejection
fraction in a substantial proportion of cases (40%). Besides, this was a prospective
study with a robust end-point (all-cause mortality or admission for worsening heart failure) and information provided/validated by two independent observers, making systematic misclassification unlikely.

Nevertheless, the association between OPG and outcome could be partly due to unspecific associations between inflammation and non-cardiovascular diseases and, as an observational study, a causal role for OPG cannot be established at this point.

CONCLUSION

Higher OPG is associated with a higher risk of death and readmission for AHF within 6 months after discharge from an index hospitalization due to AHF.

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Disclosures

The authors declare that they have no conflicts of interest.
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Table 1. Patient baseline characteristics by quartiles of serum OPG at hospital discharge.

<table>
<thead>
<tr>
<th></th>
<th>First quartile</th>
<th>Second quartile</th>
<th>Third quartile</th>
<th>Fourth quartile</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>83</td>
<td>86</td>
<td>84</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Female, %</td>
<td>44.6</td>
<td>59.3</td>
<td>56.0</td>
<td>52.9</td>
<td>0.260</td>
</tr>
<tr>
<td>Age (years), mean (SD)</td>
<td>69.9 (11.7)</td>
<td>76.0 (9.4)</td>
<td>77.1 (11.5)</td>
<td>80.0 (11.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>33.7</td>
<td>53.5</td>
<td>40.5</td>
<td>50.6</td>
<td>0.036</td>
</tr>
<tr>
<td>Ischemic etiology, %</td>
<td>41.0</td>
<td>57.0</td>
<td>46.4</td>
<td>50.6</td>
<td>0.200</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>35 (19)</td>
<td>35 (15)</td>
<td>40 (17)</td>
<td>42 (17)</td>
<td>0.033</td>
</tr>
<tr>
<td>Left ventricular systolic function, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.034</td>
</tr>
<tr>
<td>Preserved</td>
<td>33.7</td>
<td>36.0</td>
<td>45.2</td>
<td>44.7</td>
<td></td>
</tr>
<tr>
<td>Mildly depressed</td>
<td>8.4</td>
<td>3.5</td>
<td>9.5</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Moderately depressed</td>
<td>10.8</td>
<td>23.3</td>
<td>8.3</td>
<td>21.2</td>
<td></td>
</tr>
<tr>
<td>Severely depressed</td>
<td>47.0</td>
<td>37.2</td>
<td>36.9</td>
<td>30.6</td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation, %</td>
<td>47.0</td>
<td>45.9</td>
<td>47.6</td>
<td>51.8</td>
<td>0.879</td>
</tr>
<tr>
<td>NYHA class III-IV at discharge, %</td>
<td>7.2</td>
<td>16.3</td>
<td>23.8</td>
<td>27.1</td>
<td>0.005</td>
</tr>
<tr>
<td>BNP at discharge (pg/ml), median (P25-P75)</td>
<td>441 (230-1089)</td>
<td>588 (240-1565)</td>
<td>664 (300-1091)</td>
<td>927 (482-1615)</td>
<td>0.003</td>
</tr>
<tr>
<td>eGFR – MDRD (mL/min/1.73 m²), median (P25-P75)</td>
<td>56.9 (42.0-68.5)</td>
<td>47.9 (32.9-58.9)</td>
<td>47.5 (38.4-58.6)</td>
<td>41.4 (28.5-53.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>9.2 (4.0-18.2)</td>
<td>12.3 (7.4-25.4)</td>
<td>13.6 (7.4-25.8)</td>
<td>13.2 (8.3-28.9)</td>
<td>0.029</td>
</tr>
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<tr>
<td>C-reactive protein at discharge (mg/l), median (P25-P75)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of inotropic drugs during hospital admission, %</td>
<td>4.8</td>
<td>10.5</td>
<td>3.6</td>
<td>10.6</td>
<td>0.168</td>
</tr>
<tr>
<td>Beta-blocker prescribed at discharge, %</td>
<td>80.7</td>
<td>77.9</td>
<td>76.2</td>
<td>76.5</td>
<td>0.891</td>
</tr>
<tr>
<td>ACEi/ARB prescribed at discharge, %</td>
<td>83.1</td>
<td>82.6</td>
<td>76.2</td>
<td>78.8</td>
<td>0.637</td>
</tr>
<tr>
<td>Spironolactone prescribed at discharge, %</td>
<td>20.5</td>
<td>26.7</td>
<td>26.2</td>
<td>27.1</td>
<td>0.732</td>
</tr>
<tr>
<td>Loop diuretic prescribed at discharge, %</td>
<td>93.9</td>
<td>92.9</td>
<td>98.8</td>
<td>95.3</td>
<td>0.300</td>
</tr>
<tr>
<td>Statin prescribed at discharge, %</td>
<td>67.1</td>
<td>73.6</td>
<td>65.1</td>
<td>57.6</td>
<td>0.230</td>
</tr>
</tbody>
</table>

ACEi, Angiotensin converting enzyme-inhibitor; ARB, Angiotensin II receptor-blocker; BNP, B-type natriuretic peptide; eGFR-MDRD, estimated glomerular filtration rate (Modification of Diet in Renal Disease study equation); P25-P75, 25th percentile-75th percentile.

* Available for 307 patients.
Table 2. Multivariate Cox regression analysis for the association of serum OPG with all-cause death or hospital readmission due to heart failure within 6 months after discharge from the index hospitalization for acute heart failure.

<table>
<thead>
<tr>
<th>Quartiles of serum OPG</th>
<th>Overall</th>
<th>HFpEF</th>
<th>HFrEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 &lt;108.3 pg/ml</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2 108.3-146.2 pg/ml</td>
<td>1.44 (0.78-2.70)</td>
<td>3.27 (0.85-12.54)</td>
<td>0.97 (0.47-2.03)</td>
</tr>
<tr>
<td>3 146.3-184.8 pg/ml</td>
<td>1.73 (0.94-3.20)</td>
<td>1.80 (0.47-6.95)</td>
<td>1.78 (0.89-3.58)</td>
</tr>
<tr>
<td>4 184.9-506.7 pg/ml</td>
<td>2.44 (1.33-4.49)</td>
<td>4.52 (1.28-15.94)</td>
<td>1.58 (0.76-3.29)</td>
</tr>
</tbody>
</table>

* adjusted for age (continuous), diabetes (yes/no), ischaemic aetiology for heart failure (yes/no), left ventricular systolic dysfunction (preserved, mild, moderate, severe depression), New York Heart Association functional class at discharge (I-II/III-IV), estimated glomerular filtration rate at discharge (≥90, 60-89.9, 30-59.9, <30 mL/min/1.73 m²), serum B-type natriuretic peptide at discharge (continuous) and C-reactive protein at discharge (continuous). P value for interaction between OPG and preserved versus reduced ejection fraction=0.792.

Serum OPG (continuous), per 10 pg/mL: 1.05 (1.03-1.08) 1.04 (1.01-1.08) 1.06 (1.01-1.11)
Figure titles and legends

Fig. 1 Prognosis according to osteoprotegerin quartiles

Cumulative survival free of hospitalization for heart failure, within 6 months after the index hospitalization for acute heart failure, estimated by the Kaplan-Meier method, by quartiles of serum OPG at hospital discharge.